Final Minutes of the National Toxicology Program (NTP) Advisory Committee on Alternative Toxicological Methods (ACATM) Meeting

October 14, 1999

National Institute of Environmental Health Sciences (NIEHS)
Building 101
Research Triangle Park, NC

The following ACATM members were in attendance:

- Katherine A. Stitzel, D.V.M. (Chair), Procter & Gamble Company, Cincinnati, OH
- Paul T. Bailey, Ph.D., Mobil Business Resources Corporation, Paulsboro, NJ
- Elaine Faustman, Ph.D., University of Washington, Seattle, WA
- Alan M. Goldberg, Ph.D., Johns Hopkins University, Baltimore, MD
- Sidney Green, Ph.D., Howard University College of Medicine, Washington, D.C.
- A. Wallace Hayes, Ph.D., Gillette Company, Boston, MA
- Susan Hurt, Ph.D., Rohm and Haas Company, Spring House, PA
- Roger McClellan, D.V.M., Chemical Industry Institute of Toxicology, Research Triangle Park, NC
- Charles Montgomery, D.V.M., Baylor College of Medicine, Houston, TX
- Peter Theran, D.V.M., Massachusetts Society for the Prevention of Cruelty to Animals, Boston, MA

Other Meeting Attendees:

- Ms. Sara Amundson, Doris Day Animal League
- Angela Auletta, Ph.D., U.S. Environmental Protection Agency (U.S. EPA), Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C. (for Dr. Galson)
- Steven Bayard, Ph.D., Occupational Safety and Health Administration (OSHA)
- Ms. Loretta Brammell, NIEHS/NICEATM
- Mr. David Bombick, RJR Nabisco
- Betsy Carlton, Ph.D., Rhodia
- Finis Cavender, Ph.D., ILS, Inc./NICEATM
- Rodger Curren, Ph.D., IIVS, Inc.
- J.R. Fouts, Ph.D., NIEHS
- Thomas Goldsworthy, Ph.D., ILS, Inc./NICEATM
- Ms. Karen Haneke, ILS, Inc./NICEATM
- John Harbell, Ph.D., Institute for In Vitro Sciences, Inc.
- Jerry Heindel, Ph.D., NIEHS
- Mr. Patrick Herron, ILS, Inc./NICEATM
- George Lucier, Ph.D., NIEHS
- Tom Mulligan, Ph.D., Food and Drug Administration (FDA)/Center for Veterinary Medicine
- William Mundy, Ph.D., U.S. EPA, National Health and Environmental Effects Research Laboratory (NHEERL)

- Ms. Denise Sailstad, U.S. EPA
- Harry Salem, Ph.D., Department of Defense (DOD), Aberdeen Proving Ground, MD
- Phil Sayre, Ph.D., U.S. EPA
- Leonard Schechtman, Ph.D., FDA, Center for Drug Evaluation and Research (CDER)
- William Stokes, D.V.M., NIEHS/NICEATM
- Ms. Mary Beth Sweetland, People for the Ethical Treatment of Animals (PETA)
- Mr. Gary Timm, U.S. EPA
- Raymond Tice, Ph.D., ILS, Inc./NICEATM
- Ms. Heather Vahdat, ILS, Inc./NICEATM
- Marilyn Wind, Ph.D., U. S. Consumer Product Safety Commission, Bethesda, MD

Call to Order and Introductions

Dr. Stitzel called the meeting to order at 8:45 a.m. Everyone in attendance stated their name and affiliation for the record.

Welcome and National Toxicology Program (NTP) Update

Dr. Lucier welcomed everyone and expressed his satisfaction in the validation activities of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the supporting NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). Dr. Lucier stated that he had never seen an interagency activity work as well as ICCVAM and that the process is reducing the use of animals while maintaining scientific integrity.

Dr. Lucier provided an update of other activities at NIEHS and within the NTP, adding that mechanism-based toxicology has become the focus of increased attention. New research in this area has focused on microarray technology, polymorphisms, and target gene identification using microchips. He then reviewed current activities of the Laboratory of Computational Biology and Risk Assessment (LCBRA) and the NTP Center for Reproductive Toxicology. In addition, he discussed a recent workshop on the role of human exposure in the prevention of environmental disease; the Electric and Magnetic Fields (EMF) initiative; a joint project with the Environmental Protection Agency (EPA) on drinking water by-products; the creation of a new Center on Phototoxicity, jointly supported by the Food and Drug Administration (FDA), at the National Center for Toxicological Research (NCTR), and lastly, the recent meeting on the annual Report on Carcinogens.

Dr. McClellan stated that he was pleased that efforts in mechanistically based dose response modeling were increasing since the overwhelming focus has been on hazard identification. Dr. Lucier replied that this is an important step in exposure assessment. Dr. Hayes requested more information about the new Center on Phototoxicity. Dr. Lucier explained that for several years there has been an interagency agreement with the FDA to assess, at NCTR, chemicals of interest, and that in the process they have received a number of nominations to study cosmetics with potential phototoxicity. The Center was developed in response to this increased concern. The Center receives advice from an

external review board and is funded under the existing interagency agreement. The Center will officially open in late October 1999 and a number of substances for evaluation are being considered. Dr. Hayes asked if the list of substances were available for consideration and whether the methods established by the new Center would be validated. Dr. Lucier could not comment on the first question but did state that although the methods could be validated through ICCVAM, a subcommittee usually approves them.

Update on ICCVAM and NICEATM

Dr. Stokes provided an update on ICCVAM and NICEATM. Test method reviews for the Local Lymph Node Assay (LLNA) and Corrositex® have been completed and the reports of the independent peer review panel published. The LLNA Report was forwarded to ICCVAM agencies in February, and regulatory agencies will be reporting on the results of their consideration of the method at this meeting. The Corrositex® report was forwarded to ICCVAM agencies in June, and agency decisions are expected by the end of this year. He discussed the independent expert peer panels and ICCVAM working groups involved in the LLNA and Corrositex® reviews, a brief summary of each assay, and the conclusions reached by the independent expert panels. The expert peer panel for the LLNA concluded that the assay was acceptable as a stand-alone test method for allergic contact dermatitis (ACD), that some protocol modification would be needed (a suggested revised protocol was prepared by the Immunotoxicity Working Group [IWG]), and that the current guinea pig test for ACD may be needed for metals and metal compounds. With respect to animal welfare issues, the panel concluded that the LLNA provided reduction in the number of animals used compared to the traditional guinea pig assay and refinement with respect to the elimination of endpoints that lead to potential pain and distress. The expert peer panel for Corrositex® concluded that the assay was useful for tiered assessments, transportation hazard classification, and that confirmatory testing was acceptable when deemed necessary.

Dr. Stokes then explained the ICCVAM test method evaluation process and listed the relevant regulatory agencies and international organizations that were considering the two alternative assays.

With regard to the methods currently under review, Dr. Stokes discussed the current status of the Frog Embryo Teratogenesis Assay – Xenopus (FETAX), stating that a draft comprehensive background review document (BRD) has been prepared by NICEATM and that an expert panel meeting is scheduled to take place in May 2000. Dr. Stokes then summarized the current objectives for the FETAX expert panel meeting. These included: evaluating the current validation status of the FETAX protocol, recommending an optimized protocol, identifying further validation studies needed to characterize the usefulness of FETAX, and identifying additional research and development efforts that might further enhance the effectiveness of FETAX.

Next, the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) program was summarized. Results of the evaluation show that the MEIC approach may be useful, that

the proposed test battery will need to address regulatory need for an estimate of LD50 rather than LC50 values, and that additional methods are necessary to address toxicokinetics (ADME) and receptor activities. For MEIC, ICCVAM has recommended that a workshop to identify future directions with respect to the program and objectives be held.

Dr. Stokes listed test methods that are under consideration for future review. These include the revised up-and-down procedure for acute oral lethality testing (Organisation for Economic Co-operation and Development (OECD) Guideline 425), endocrine disruptor screening and testing methods, transgenic models for carcinogenicity, *in vitro* dermal corrosivity assays, and the HCE-7 ocular irritancy assay.

The Endocrine Disruptor Working Group (EDWG), consisting of 23 scientists from nine agencies, met for the first time on June 21, 1999, and provided comments on the proposed OECD rat uterotropic assay.

He also announced that the ICCVAM Test Method Submission Guidelines had been revised, and that the guidelines are available both on the NICEATM website (http://iccvam.niehs.nih.gov) and upon request from NICEATM. The revised guidelines provide a format for background review document (BRD) submission, describe data and information needed to assess a test method's current validation status, and provide a basis for decision on standardized protocols and validation study designs.

Dr. Stokes closed by discussing ICCVAM's interactions with the OECD, which include the EPA Endocrine Disruptor Standardization and Validation Group and the OECD Guidance Document on Humane Endpoints in Toxicity Testing. He added that ICCVAM/NICEATM was represented by ten platform and poster presentations at the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, which took place in August/September in Bologna, Italy.

Dr. Goldberg inquired as to whether OECD Guideline 425 would be considered a stand-alone guideline and if it would be compared with Guideline 401. Dr. Auletta replied that Guideline 401 is expected to be deleted by the end of the year 2000, and that Guideline 425 is being revised to provide the dose-response data that is required by EPA in making its regulatory decisions. Dr. Green asked about the expected time frame for revision to 425, to which Dr. Auletta answered that the original deadline was October 1, but that it had been postponed until later this year. Dr. Stokes anticipated that NICEATM would fast-track this review effort while working closely with the statisticians involved in computer simulation modeling.

Dr. Hurt asked if the FETAX expert panel would be asked to determine whether the assay is a short-term predictor of toxicity and if other *in vitro* assays will be considered. Dr. Stokes replied that the panel would be evaluating the current validation status of FETAX, including its current and potential usefulness and limitations, and what studies are needed to further optimize the assay for its proposed use(s). Dr. Lucier suggested that

a comparison between FETAX and other *in vitro* methods for assessing teratogenicity would be useful.

Regulatory Agency Processes for Consideration of ICCVAM Test Method Recommendations: Acceptance Consideration of the LLNA

Food and Drug Administration (FDA)

Dr. Schechtman reviewed the FDA process for responding to ICCVAM recommendations. He summarized the purpose and product responsibilities of each Center within the agency and their respective representation for ICCVAM-related activities. The Office of Testing and Research (OTR) serves as the lead office for FDA representation to ICCVAM and is responsible for coordinating the dissemination of information throughout FDA, between FDA and ICCVAM, and to the public sector. OTR also assists other Centers in implementing ICCVAM-recommended test methods. Whereas the different FDA product centers respond to ICCVAM recommendations as a function of the products they regulate, the National Center for Toxicological Research (NCTR) and the Office of Regulatory Affairs (ORA) do so in a different capacity. NCTR is not obliged to develop a separate process for the review of ICCVAM recommendations, as it does not function in a regulatory capacity *per se*. Among its various activities, NCTR conducts research in support of the regulatory responsibilities of the other FDA Centers and participates in a consultative manner with ICCVAM. ORA works closely with the Centers in determining what test methods may be needed for inspection/surveillance purposes and will adopt ICCVAM-endorsed methods recommended by a given Center as applicable to its product domain.

A general description of the process used by FDA in responding to ICCVAM recommendations was presented. The recommendations of ICCVAM are reviewed within each center by a designated technical core unit. The test method is reviewed for its ability to serve as a regulatory tool, potential product areas of use, and technical and practical limitations. The technical unit then forwards its recommendations to the specific division, division director, or coordinating committee where the method is considered with respect to general utility and regulatory applicability for Center-regulated products. A center spokesperson informs ICCVAM, via the FDA liaison, as to the center's decision regarding applicability of a given ICCVAM-recommended method within that center. The center spokesperson is also responsible for providing for education of the regulatory review staff on the technical basis, utility, limitations and application of the method, and is also responsible for information dissemination both internally and to the public. Information dissemination at FDA flows from the Center's offices, divisions, and appropriate committees to the regulated community and other constituents. The public is notified of the Center's anticipated use of the test method via one or more of various means such as publications, presentations at open meetings, guidance documents, guidelines, regulations, and Federal Register (FR) announcements and internet web sites. Center regulatory units are notified via internal seminars, workshops, and training courses as well as by participation in meetings, conferences and panels, and intranet web sites. The ultimate decision to implement an ICCVAM-

recommended test method is based on whether the method satisfies a center's scientific criteria in meeting its regulatory commitment to establish product safety and efficacy.

Dr. Schechtman stated that the overall conclusion of the FDA is that the LLNA is an acceptable alternative to the Guinea Pig Maximization Test (GPMT) for hazard identification of strong/moderate contact chemical sensitizing agents. He indicated, however, that the applicability varies by Center. Although each center had certain reservations, not altogether different from those stipulated by the ICCVAM Peer Review Panel, Overall, CDER, the Center for Biologics Evaluation and Research (CBER), and the Center for Food Safety and Applied Nutrition (CFSAN) found the LLNA to be acceptable for use as an alternative to the GPMT. The Center for Veterinary Medicine (CVM) also found the LLNA acceptable as an alternative to the GPMT, but has no need to include the LLNA in its testing guidelines. On the rare occasion when a veterinary drug requires hypersensitivity testing, preference will be given to use the most appropriate animal. The Center for Devices and Radiological Health (CDRH) will accept the LLNA with the caveat that positive and negative controls appropriate for testing extracts of materials/devices must be included, and that both auricular lymph nodes per mouse are sampled. CFSAN is currently evaluating the method's applicability for detecting allergic contact dermatitis mediated by food and color additives and cosmetics.

Dr. Goldberg asked if it would be possible to track the number of GPMT tests that were used compared to the LLNA in a given time frame. Dr. Schechtman replied that such information should be possible to collect. Dr. Harbell questioned whether reviewing an assay on an individual Center basis was repeating the work already conducted by the ICCVAM expert panel. Dr. Schechtman replied the Center reviews consist of taking the data and recommendations that were generated through the ICCVAM process and disseminating it for comment among the staff. This process is viewed more as a Center-specific focusing process rather than a re-evaluation. Dr. Schechtman further stated that the FDA was very pleased with the efficiency of the Centers' representatives, the energy and effort that went into the centers' review process, and their quick responses to this important issue. He expects that the process will become even more efficient as it's use increases.

Dr. Green congratulated the FDA for their review process and agreed that decentralizing the decision-making process allows each Center to evaluate the assay based on their individual regulatory responsibilities. He asked how inconsistencies between Centers regarding the acceptability of a method would be addressed. Dr. Schechtman replied that each Center functions as an independent entity, and that while consistency within a center is expected, consistency within the entire agency is not necessarily possible.
Dr. Stokes announced that the database for methods reviewed by ICCVAM continues to expand. NICEATM has received six additional publications from the LLNA sponsors since the initial submission was provided. These publications address many of the purported assay limitations and will be posted on the ICCVAM website.

U.S. Environmental Protection Agency (EPA)

Dr. Auletta presented the EPA position on the regulatory acceptance of the LLNA. Dr. Auletta reviewed the various EPA programs - solid waste, air, pesticides, and toxic substances - that have oversight for toxicological testing. She explained that ICCVAM has EPA representatives from the Offices of Pesticides, Pollution Prevention and Toxics, and Research and Development. Working group members are assigned on an *ad hoc* basis, and the evaluation of ICCVAM reports is carried out by the Office of Science Coordination and Policy. Dr. Auletta stated that consensus was reached by all of the aforementioned offices. The EPA finds the LLNA acceptable as a free standing test for contact sensitivity in the pesticide and toxic substances programs and is the preferred method of testing materials where there are no reservations concerning its use. The EPA states that the GPMT should be retained for those cases where the LLNA is not warranted.

Dr. Bailey asked what the status was for the LLNA with respect to the OECD. Dr. Auletta replied that the LLNA guideline developed by the United Kingdom for submission to the OECD was withdrawn. The guideline will be resubmitted, at which point it will be submitted to the member countries for review. She was not able to say when the revised LLNA guideline will be submitted. Dr. Stokes added that the LLNA guideline also needed to be revised consistent with changes in the way the guideline was organized before it was submitted.

Dr. Hurt asked how EPA planned to bridge between scientific acceptance and administrative acceptance. Dr. Auletta stated that all of the scientists were required to bring administrative supervisors to the meetings to ensure that there would be administrative acceptance as well. Dr. Green then asked how EPA's decisions would be made public. Dr. Auletta replied that the appropriate procedure had not yet been finalized but that, at a minimum, an appropriate statement would go into the FR. Dr. Faustman suggested that the agencies should be given the charge of raising awareness about newly accepted assays and that sponsoring training sessions at professional meetings might be one approach. Dr. Auletta stated that this might be better handled through ICCVAM. Dr. Lucier replied that ICCVAM does not make announcements regarding the agencies' formal acceptance of the methods. Dr. Schechtman suggested that relying solely on the agencies might not be the most effective method of disseminating information and that ICCVAM should provide continuing education, perhaps at professional meetings, to educate those who will be exposed to the new methods. Dr. Hayes commented that information dissemination by ICCVAM was a good idea, but that the individual agencies should be responsible for informing the regulated community about methods that are acceptable under their regulations. He added that if agencies took this step, then the educational programs would be more successful. Dr. Stitzel stated that the ACATM members were pleased with how the EPA had completed their evaluation of the LLNA.

U.S. Consumer Product Safety Commission (CPSC)

Dr. Wind presented the CPSC process for acceptance of ICCVAM test method recommendations. Dr. Wind began by explaining that under the Federal Hazardous Substances Act (FHSA), manufacturers are responsible for the evaluation of the acute and chronic toxicity of a product and for the appropriate labeling needed to address hazards associated with handling and use. Under FHSA, classifications of hazardous substances include toxic, corrosive, skin or eye irritant, strong sensitizer, flammable or combustible, and generates pressure. Dr. Wind also provided the LC_{50} and LD_{50} levels that designate highly toxic substances as defined in the FHSA, and toxic substances as defined in the Code of Federal Regulations (CFR). She listed definitions of the terms corrosive, irritant, and strong sensitizer, as designated by CPSC. She stated that with the exception of the term 'highly toxic,' the FHSA defines hazards in terms of their effect on humans. She pointed out that available human data takes precedence over animal test results. Dr. Wind stated that the LLNA was not reviewed by the CPSC because it will not be used for labeling under the FHSA. She explained that labeling is not required under FHSA for ACD and that the definition of a strong sensitizer specifies the frequency and severity of a reaction in humans. Corrositex® will be evaluated by the CPSC as it tests corrosivity, which requires labeling under FHSA. She stated that a FR notice would be published with their response. Dr. Wind added that the CPSC does not require specific tests, rather they will recommend a method per its acceptance by an internal review within the commission.

Dr. Theran asked when a conclusion regarding Corrositex® might be anticipated. Dr. Wind responded that a conclusion should be reached before the end of the year. Dr. Goldberg asked Dr. Wind if she had any expectation as to what the conclusion would be. Dr. Wind replied that she expects that the CPSC would adopt the recommendations of the ICCVAM Corrositex® expert peer panel. Dr. Theran suggested that the CPSC use acceptance terminology similar to that used by the FDA, stating that the assay is an appropriate test in some testing circumstances. He added that a directive from the agency stating that an assay should be used unless it is not applicable would be the most effective method for encouraging its use. Dr. Wind replied that the CPSC does not recommend what method should be used; rather, they deal with whether the labeling is appropriate.

Occupational Safety and Health Administration (OSHA)

Dr. Bayard presented OSHA's position on the use of the LLNA under the Hazard Communication Standard (HAZCOM), which was established in 1983. Dr. Bayard began by explaining the role that OSHA plays with respect to occupational safety and health, the distribution of staff members throughout the country, and the scope of HAZCOM. The responsibilities of the chemical manufacturers and importers is to provide appropriate warning of the hazards of the chemicals that they are producing or importing. The responsibilities of the employers is to provide information to those employees who are exposed to hazardous chemicals. He then listed the products/agents to which HAZCOM does not apply and the criteria by which OSHA judges the hazardous nature of a chemical. These criteria include chemicals: regulated by OSHA, with a

Threshold Limit Value (TLV) published by the American Conference of Governmental Industrial Hygienists (ACGIH), and found to be potential or confirmed carcinogens by either the NTP or the International Agency for Research on Cancer (IARC).

With respect to mixtures, HAZCOM determines the hazard potential first by using data derived from a test of the whole mixture. If this data are not available, the chemical manufacturer, importer, or employer may use any available scientifically valid data to assess the hazard potential of the mixture. HAZCOM states that if there is no whole mixture data available, the assumption should be made that the given mixture has the same health hazards as those components that comprise one percent or more by weight or volume of the mixture. For carcinogens, the concentration is one-tenth of one percent or greater. Dr. Bayard also described the Hazard Communication Program, which consists of a written plan describing how the requirements of HAZCOM will be carried out in the workplace. The three major means of communicating this information include labels, training, and Material Safety Data Sheets (MSDS), the latter of which can be located on the internet at http://www.phys.ksu.edu/~tipping/msds.html.

Dr. Bayard closed by summarizing OSHA's position on the LLNA and Corrositex® assays. He stated that OSHA has no authority to require testing and that HAZCOM requires manufacturers and importers to perform hazard evaluations that consider all available scientific evidence. This evaluation may be based solely on the information currently available in the scientific literature and the hazard determination must be kept current. Dr. Bayard also stated that short-term tests have not been specifically addressed in either the HAZCOM final rule (59FR.6126) or the preamble discussion. However, Appendix B of the rule allows the use of studies for determining potential hazard if the study is designed and conducted according to established scientific principles. The results of such studies must contain statistically significant conclusions with respect to health effects. OSHA's position on *in vitro* studies is that they are "useful information, but not definitive finding of hazards." Therefore, OSHA regulations do not encourage the replacement of *in vivo* studies. With respect to the LLNA, OSHA believes that the independent review and validation process as established by ICCVAM is scientifically sound as described in Appendix B of HAZCOM. Therefore, OSHA will accept any positive study determined using the LLNA as an indicator of ACD potential, but does not consider negative results to have ruled out the possibility of a hazard. OSHA will inform the regulated community of its decision via a FR notice and will provide compliance officers with a copy of the ICCVAM report. As for Corrositex®, OSHA will defer to the DOT's regulation regarding its use. DOT allows for the use of Corrositex® for determining the corrosive potential based on an issued exemption. Dr. Bayard stated that OSHA would continue to accept the assay as long as the DOT does.

Dr. Stitzel asked if OSHA does not accept a negative test because of a policy where positive responses are given weight over negative ones. Dr. Bayard replied in the affirmative. Dr. Theran stated that, in some cases, scientific evidence demonstrates as well that an *in vivo* study is useful but not definitive. Therefore, he asked for clarification as to why there would be a policy to categorically assign more weight to *in vivo* tests. Dr. Bayard replied that this is part of the OSHA policy of standards for chemical

evaluation. He also stated that the policy is based on a weight-of-evidence approach and that *in vitro* studies have not carried the same weight as *in vivo* studies. Dr. Theran said that he found this statement difficult to justify scientifically and asked that OSHA look into this further. Dr. Goldberg stated that there are situations where *in vitro* tests provide a definitive answer, such as pregnancy tests. He stated that he agreed with Dr. Theran that the wording presented by OSHA is disturbing. Dr. Auletta added that OSHA is erring on the side of caution and that this is why they do not accept negatives. Dr. Schechtman asked how OSHA would handle equivocal results. The reply was that they would be considered as positive results since the issue is worker protection. Dr. Stitzel congratulated OSHA on accepting the LLNA and Corrositex®

Dr. Stokes indicated that, in addition to the four Federal agencies presenting at the meeting, responses had been received from the Department of Defense (DOD) and other agencies that are not involved with drafting regulations

Public Comment

Ms. Sarah Amundson from the Doris Day Animal League requested that the ACATM help to ensure that the regulated community and the public are aware that the agencies are committed to the ICCVAM process. She hoped that the ICCVAM Authorization Act, brought forth by Senator Dewine, would receive broad-based senate support and that it would be helpful if ACATM would provide support via telephone calls and letters. Dr. Faustman stated that there was some concern with the requirement in the Authorization Act that agencies need to consider methods and reply within 180 days. She queried if this time frame was considered workable by the various agencies. Ms. Amundson replied that this language was chosen based on other statutory regulations and was not meant to serve as an absolute deadline, but as a guideline. Dr. Schechtman made the comment that when a time limit is codified, then agencies are legally bound to act within that time period. He suggested that the statute be reworded to provide increased flexibility. Dr. Auletta agreed with Dr. Schechtman and pointed out that wording in the proposed Act allows for 180 days to accept the method, not to review it.

Update on EPA Standardization and Validation Task Force Activities/OECD Endocrine Screening and Testing Validation Efforts

Mr. Timm presented information from the U.S. EPA Endocrine Disruptor Screening Program. He presented a flow chart of how chemicals progress through the screening program and provided an overview of the 71 specific recommendations that had been received from the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC). Mr. Timm also presented plans for developing a tiered testing strategy, which consists of a primary tier for detecting potential endocrine disruption and a secondary tier for the confirmation and characterization of endocrine effects. He provided the proposed screening batteries for each tier as well as two additional examples for tier 1.

Mr. Timm also reviewed the scientific and budgetary challenges that are facing the EPA. The endocrine disruptor issue is a highly visible, major priority for the agency, but the number of assays requiring development and validation is unprecedented in regulatory history. The EPA views the ICCVAM process as being a substantially expanded formal scientific process for validation. He explained that the budgetary issues include the fact that EDSTAC budget assumptions are no longer valid. The regulated community is on record as stating it will play a minimal role in funding standardization and validation. Contract laboratory and inter-agency funding partnerships have not materialized. International activities are currently limited to two assays. Furthermore, the ICCVAM principles and process were not originally anticipated by EDSTAC.

He provided two validation options for the chemicals. The first option is the original Standardization and Validation Task Force (SVTF), which involves the validation of a battery of assays for a block of chemicals. This option calls for three laboratories performing 11 Tier 1 screening assays on 16 chemicals and three laboratories performing five Tier 2 tests on six chemicals. The anticipated cost is $55 million. This plan would be both time and resource consuming, but EPA considers that it is scientifically rigorous and meets all ICCVAM principles. The second option is a modified validation where a single assay, rather than a battery of assays, is validated. This process would cost between $15 and $30 million, but is less rigorous than option 1, and is therefore criticized by industry. The advantages of using this option are that it is less time and resource intensive while still meeting the ICCVAM guidelines and exceeding historical regulatory guidelines for developmental processes. The EPA has made the decision to proceed with the second validation option since it balances scientific integrity and available resources. He explained that human health-related assays are the highest priority at the EPA and that the timeframe for completion is two to three years. The second priority is the validation of ecotoxicological assays, which should be completed within three to six years. He described the High Throughput Pre-Screen (HTPS) demonstration that was used to evaluate the feasibility of using reporter gene assays. This demonstration showed low sensitivity and high variability and was, therefore, deemed unsuccessful. However, this lack of success should not rule out the successful use of other assay systems and approaches. Mr. Timm presented the EPA's decision regarding HTPS and *in vitro* assays. The decision was made to use models rather than HTPS for priority setting of Phase 1 chemicals, focus would be on pesticide-active ingredients and the formulation of inert ingredients for Phase 1, and HTPS would be deferred until Phase 2. EPA will also initiate a challenge program for *in vitro* technologies and develop a general guideline for bench assays for estrogen and androgen receptors in tier 1. The *in vivo* assays that will be developed and standardized by the EPA include the female and male pubertal development assays, the frog metamorphosis assay, and the fish reproduction screening assay. Mr. Timm also described alternative assay research, including the trout estrogen receptor binding assay, transcriptional activation assays, and a tissue response assay.

Mr. Timm closed by presenting the activities of the OECD endocrine disruptor testing and assessment workgroup, including its work on standardizing/validating the uterotropic, Hershberger, and 28-day repeat dose studies (OECD 407). He also presented questions regarding standardization and validation, including how much validation is

necessary before an assay can be used for regulatory applications and whether validation should continue after regulatory implementation.

Dr. Faustman requested that Mr. Timm elaborate on the problems within the HTPS in regard to low sensitivity, etc. She asked if a statistical analysis had been performed or if it was obvious that things were not working. Mr. Timm replied that a statistical analysis was performed but that it was also clear without this analysis that assay predictivity was poor.

Dr. Goldberg asked if the estrogen receptors were divided into subtypes. Mr. Timm replied in the negative, stating that this was a screening phase to merely get an indication of endocrine disruptor activity. He also said that they are looking for a better indicator of activity and that it is not necessary to separate subtypes for the reporter gene assay. With respect to cell free receptor assays, Mr. Timm stated that no work had been carried out on these as of yet because EDSTAC wants the industrial community to take a known set of chemicals and self-validate cell lines that they select. Dr. Goldberg pointed out that the focus of the endocrine issue is to evaluate for potential human health effects, limited to pesticides and estrogen. Mr. Timm replied that the statutes said to focus on human health, pesticides, and estrogen but that at the first EDSTAC meeting, it was decided that this was too narrow of a focus. Anti-androgenic effects are just as important as estrogenic effects; that the best evidence of health effects are in wildlife species, not humans; and there is a need to go beyond pesticides into more broad categories. The decision was made not to focus on human health alone since the EPA is responsible for taking into account human health and wildlife issues. However, these limitations are imposed by time and money constraints.

Dr. Faustman said that she was curious as to what EPA was looking for with respect to false positive and false negative rates for endocrine disruptors. Mr. Timm stated that false positive and negative rates were considered by EDSTAC but that no specific criteria were recommended by EDSTAC or the EPA. Dr. Faustman asked how positive and negative chemicals were chosen for the tests already conducted, and suggested that ICCVAM could assist the EPA in establishing chemical selection criteria. Dr. Stitzel stated that she didn't know the names of all the chemicals selected, but assumed that the EPA would not have included many negatives. She added that a second tier assay is expensive and that thought needs to be given to how many compounds are needed to produce statistically sound results. Dr. Montgomery suggested that a way of approaching this would be to establish a relational database and that gene structure activity relationships should be included in an analysis of chemical structure activity relationships. He stated that it is reasonable to use this tool as a way to make decisions as to which chemicals to test. Dr. Montgomery asked what the progress was to date on the relational database. Mr. Timm responded that the database was originally designed to be a more robust version that included the capability of creating models but that it had been scaled back for use in priority setting only. He explained that NCTR has a whole family of models for 220 compounds and that the EPA will look to NCTR and the EPA laboratory at Duluth for this information.

Dr. Lucier commented that there have been problems historically with the evaluation of estrogen receptors. A key concern is that they bind to a variety of chemicals without regard to known structure-activity relationships. Weak-binding steroidal estrogens are an example. There are many mechanisms in addition to classical receptor mechanisms and it is unclear how many of these need to be considered. There are a lot of significant obstacles that need to be overcome before the establishment of HTPS and that a considerable narrowing of the focus needs to take place. He suggested that the focus should be on looking at one mechanism at a time. He concluded that for the EPA to progress, they would need input from ICCVAM as well as from others.

Dr. Stitzel stated that she believed that it would be worthwhile to set up advisory groups and to use their expertise to address questions that have been raised during this meeting. She said that agencies have created tests in the past, but never in such a short time frame. She felt that more input was needed, and suggested that the EPA work with ICCVAM. Mr. Timm replied that the EPA plans on continuing dialogue with Drs. Lucier and Stokes to deal with these issues. A full ICCVAM review was not planned because it would duplicate much of the EPA review process. Dr. Stitzel strongly suggested that the ICCVAM submission guidelines be considered so that the most important issues in validation would be addressed. Some steps seem to be cost and labor intensive but are needed to ensure scientifically sound evaluation. Dr. Hurt added that the ICCVAM process could help with the identification of steps for development as well as the separation and validation of the whole battery as individual tests. Dr. Stitzel suggested that ICCVAM might assist the EPA in establishing a background review document prior to initiating validation studies to ensure that they addressed all the essential points in the validation study design. Dr. Stokes added that ICCVAM has established an Endocrine Disruptor Working Group (EDWG) with 23 scientists from nine agencies and that this group could provide feedback and review proposed validation studies. He stated that not all of the members are experts in endocrine disruption, but have expertise in validation and regulatory testing methodology. Dr. Stitzel added that other scientists could be consulted as well. Discussion followed on the need to validate both *in vivo* and *in vitro* components. Dr. Hurt stated that it has been common practice for an agency to semi-standardize a test method and then to use the assay for regulatory decision making. Avian endpoints were cited as an example of an *in vivo* test. The results of these tests are both statistically significant and biologically relevant. However, it is more difficult to determine if an *in vitro* endpoint is biologically relevant. Mr. Timm replied that the EPA wanted both *in vitro* and *in vivo* assays. Dr. Hurt commented by saying that validation is more critical for *in vitro* assays. Dr. Stitzel interjected that, depending on the purpose of the test, validation of *in vivo* assays can be equally important.

Overview of the Multicenter Evaluation of In Vitro Cytotoxicity (MEIC) Test Program

Dr. Harbell from the Institute for In Vitro Sciences, Inc. (IIVS) gave an overview of the MEIC test program. He explained that Dr. Bjorn Ekwall and his associates have proposed the basal cytotoxicity concept that approximately 80% of chemical-induced systemic toxicity results from disruption of basic cellular processes common to most cell

types. Fifty chemicals were selected for evaluation based on the availability of human systemic toxicity data from accidental and overdose exposure. Investigators were invited to purchase the chemicals and to perform *in vitro* assays to identify the concentration that inhibited the toxicity endpoint by 50% of the control level (i.e., the $IC_{50)}$. These data were then submitted to MEIC for evaluation. Individual laboratories selected the protocols, most of the assays used dose as the independent variable, and replicate tests were not necessarily performed. MEIC then, through the process of principle component analysis and multivariate partial least squares modeling, determined an optimal assay battery. MEIC also selectively removed twelve of the chemicals, thus limiting the analysis to 38 chemicals. A battery of three of these human cell line tests was found to be highly predictive (R^2 = 0.77) of the human lethal blood concentrations (LC50) of chemicals. The prediction increased markedly (R^2 = 0.83) when a simple algorithm based on the knowledge of passage across the blood-brain barrier was used to adapt *in vitro* to *in vivo* concentrations. The battery involved four endpoints and two exposure times (protein content/24 hours; ATP content/24 hours; inhibition of elongation of cells/24 hours; pH change/7 days). Prediction was better than the prediction of human lethal doses by rat and mouse LD50-values (R^2 = 0.65). No prediction models have been developed.

Dr. Harbell provided an example of how the process works using the Neutral Red Uptake (NRU) Bioassay in Normal Human Epidermal Keratinocytes (NHEK) cells. Based on the results obtained, there seems to be a strong correlation between *in vitro* cytotoxicity and LC_{50}, that exposures of 24 hours or greater are better, and that human cells may be more appropriate. It was difficult to select the optimal battery since not all chemicals were tested in all assays. In an effort to strengthen the analysis, replicate tests with defined protocols were performed in several laboratories, statistics should be performed on individual tests with the full complement of chemicals tested, and the human database was increased.

Dr. Faustman stated that slopes of dose-response curves can be quite different and suggested using the slope in decision-making in addition to LD50 values. Dr. Harbell felt that the suggestion was justified in that some compounds are expected to have a wider range of activity (e.g., enzyme inhibitors). Dr. Faustman indicated that the current MEIC database includes very few compounds that require metabolic activation. Dr. Harbell replied that some of the compounds tested do require activation to induce a toxic effect. He added that there is not a direct correlation between test results and LD_{50} values but that MEIC has proposed a battery for detecting acute systemic toxicity. The cytotoxicity test would be one part of that battery.

Dr. Hurt asked about the relationship between NRU50 and IC50s. Dr. Harbell explained that NRU50 is the IC50 for the Neutral Red Uptake Assay. He stated that endpoints measured in other assays included total protein in hepG2, total protein in Chang liver cells, and the ATP concentration in HL60 cells, and that each has a corresponding IC50 value. Dr. Stitzel asked if the difference between LC_{50} and LD_{50} is whether absorption and metabolism were measured. Dr. Harbell replied in the affirmative stating that Dr. Ekwall discusses these considerations in his papers.

Potential Use of *In Vitro* Cytotoxicity Tests to Predict Acute Oral Lethality of Chemicals

Dr. Curren from IIVS presented potential future applications of the MEIC findings. Dr. Curren discussed the three basic assumptions underlying the MEIC findings. The first assumption reiterates the MEIC hypothesis, as explained earlier by Dr. Harbell, that most common causes of toxicity are due to basal toxicity. The second assumption is that (1) the minimal effective dose (MED) is a function of minimal effective organ concentration (MEOC) (e.g., MED = f[MEOC)]); (2) minimal effective cell concentration (MECC) *in vitro* is representative of MEOC (e.g., $MECC_{in\ vitro}$ = MEOC); and MED is a function of MECC *in vitro* and biokinetics (e.g., MED = $MECC_{in\ vitro}$ [A + B + D + E]). The third assumption is that the MEOC is equal to the minimal effective blood concentration (MEBC) across the blood brain interface e.g. MEOC = MEBC ($D_{blood/brain\ interface}$).

Based on his review, Dr. Curren stated that there does seem to be a strong correlation between concentrations of test material causing cytotoxicity *in vitro* and human lethal serum concentrations, but that the strength and universality of the data associations must be further explored. He identified some of the problems with the conclusions, such as the fact that studies were not conducted under controlled conditions and were generally conducted without replicates, that statistical evaluations were often carried out on groups of tests rather than individual tests, and that many methods did not evaluate all 50 chemicals, which could influence direct comparison of the r values.

Dr. Curren presented the MEIC proposed test battery, which consists of three human cell tests: the HepG2 protein content, the HL-60 ATP content, and the Chang liver morphology tests. The confounding factors for these selections range from varying results among the three different HepG2 assays submitted to MEIC, the presence of HeLa markers in Chang Liver cell lines, and the reporting of only one data value per compound thus providing no information on variability. In addition, all three cell lines are transformed; there are no normal, diploid cells suggested for the battery. He suggested that the next steps should be to choose the right battery and prediction model by further evaluation of 3-4 candidate human cell types for reproducibility and reliability, and to analyze the results with respect to the need for additional cell types and the effect of chemical passage through the blood/brain barrier.

Dr. Curren concluded by discussing what is really needed to replace LD50. There is agreement that the ultimate goal is the prediction of lethal dose to humans. He recommended that there needs to be a compilation of a gold standard set of human systemic toxicity data with corresponding chemicals as well as the acceptance of a level of predictivity for a battery of *in vitro* assays similar to that used for current animal models. Dr. Curren suggested the development of an optimization process that utilizes existing knowledge and current research efforts in addition to new programs. He provided a list of resources that are available to assist in a coordinated effort to work through these issues.

Dr. Stitzel stated that Dr. Stokes would like the advice of ACATM as to how ICCVAM should respond to the MEIC proposal and that he would be presenting the questions raised by ICCVAM.

Dr. Stokes stated that preliminary discussions had taken place at the August ICCVAM meeting regarding how to progress with the consideration of MEIC for predicting LD50 values. The committee prepared a preliminary list of objectives for a workshop to identify what the next steps should be. These objectives are to identify: critical information needed from an *in vitro* test battery to meet regulatory testing needs; needs for additional research and method development; methods that should be further optimized and evaluated; and reference chemicals with appropriate human and animal data that could be used in development and validation studies.

Dr. Stokes stated that he was interested in comments from the ACATM panel based on the presentations seen in this meeting as well as suggestions for other aspects of a workshop.

Dr. Stitzel asked if the proposed response is to coordinate a workshop to assess MEIC. Dr. Stokes replied that it was and commented that *in vitro* test data could help to identify a starting dose to reduce the number of animals used in acute toxicity tests.
Dr. McClellan asked about the possible goals of the workshop. One goal is through MEIC where an empirical approach has been used to identify *in vitro* assays for predicting acute death in terms of *in vivo* data. He noted, however, that there were five orders of magnitude difference for the *in vitro* data and two orders of magnitude difference for the *in vivo* data when discussing absorption kinetics, body burden, etc. He stated that in trying to understand the relationship between five and two orders of magnitude, there is a need to step back and identify what is trying to be accomplished. Dr. McClellan then stated that biologically based models for acute toxicity need to be questioned, and that it would be tough to mimic oral ingestion situations. Dr. Hayes added that it would be tough to do this for dermal situations as well. Dr. Lucier stated that this was a good point and that ICCVAM needed to look carefully at how to proceed with this. It was generally concluded that more thought needed to be directed toward determining what the workshop is meant to accomplish. Dr. Lucier closed the discussion by stating that ICCVAM would carefully evaluate the most appropriate way to proceed.

Dr. Stitzel the opened the floor for public comment.

Public Comment

Ms. Sweetland read a statement on behalf of People for the Ethical Treatment of Animals (PETA). The statement expressed PETA's concern that the EPA is requiring animal tests for the endocrine disruptor screening program without requiring that the tests be validated for their relevance. She went on to say that these test batteries should meet at least the same standards required of alternative test methods submitted to ICCVAM. PETA is outraged that HTPS, an EDSTAC recommended non-animal screen, seems to be a dead issue at EPA and that it now appears as though the EPA will spend the remaining $4

million originally allocated for the non-animal screen on further development of animal tests. She stated that among the tests EPA is pushing forward are those that require an enormous number of animals be killed for a single endpoint, such as the Hershberger assay. She stated that this is especially troubling to PETA as Dr. Ian Purchase presented a paper to the joint SAP/SAB subcommittee last spring that showed that decreasing the specificity of the screening tests by 20% would save the lives of 25,000 animals for every 1000 chemicals tested. The paper also showed that if the EPA's screening program were to go forward as currently designed, it would result in the deaths of up to 150 million animals. She stated that members of the SAP have told the EPA that it is reprehensible that the agency has not included a single *in vivo* assay to be developed and used as a single tier I test. She added that at the 3rd World Congress Meeting in Bologna, Italy, international scientists denounced the EPA's endocrine disruptor program. She closed by expressing PETA's disappointment that they have not been involved in meetings relating to the development and validation of endocrine disruptor screening tests despite their attempts to be included on all related e-mail lists and the well known fact that they are interested in this issue.

No other public comments were made.

Dr. Stitzel commended the agencies that reviewed and made decisions regarding ICCVAM-recommended assays and thanked ICCVAM for their efforts as well.
Dr. Stokes thanked the committee and stakeholders involved in the process.
Dr. McClellan commented that if would be helpful if agencies could report to ACATM on a regular basis regarding the procedures and how they have been used. Dr. Theran added that the Massachusetts Society for the Prevention of Cruelty to Animals is pleased with the efforts of ICCVAM and that they have given Dr. Stokes their Veterinarian-Of-The-Year Award.

Adjournment

The meeting was adjourned at 4:00 p.m.